ABSTRACT: In first part of this paper an overview is given about glucomannans sources, characterisation and use for healing of human diseases. In the second part an experimental study is presented. The study is focused on assessing the contribution of the in vivo antioxidant properties of Candida utilis cell-wall glucomannan (GM) on its anti-arthritic effect. For this purpose not only biochemical methods were used but also chemiluminiscence measurements were performed in whole blood and in selected tissues, as spleen and hind paw taken from arthritic rats treated with GM in two doses (7.5 mg/kg and of 15 mg/kg body weight) daily per os during 28 experimental days. Additionally, the functionality of neutrophils was evaluated by flow cytometry. An original approach was the determination of CoQ levels in mitochondria of skeletal muscles in AA treated by GM. The anti-arthritic effect of GM in adjuvant arthritis was evidenced using evaluation with clinical parameters – hind paw volume and body weight of experimental Lewis rats. GM in vivo antioxidant activity has a large impact on this effect, both in the whole system and locally. The local effect of GM was primarily exerted in the hind paw tissue and mitochondria of skeletal muscles. As to the intensity of GM effects observed for the tested doses of 7.5 and 15 mg/kg b.w., no significant difference was manifested; however the lower dose was more effective concerning the number of parameters affected. The results suggest that Candida utilis cell-wall GM is a potential immunomodulative agent in treating exaggerated neutrophilic inflammation accompanying arthritis and thus GM seems to be beneficial for rheumatoid arthritis therapy.

KEY WORDS: Antirheumatic therapy, Arthritis, Candida utilis, Dietary supplement, Glucomannan, Natural polysaccharides, Oxidative stress, Yeast

INTRODUCTION

Rheumatoid arthritis and the need for new antirheumatics.

Rheumatoid arthritis (RA) is a systemic, autoimmune disease with not entirely clear etiology. It is characterized by chronic inflammation of the synovial membrane and by the destruction of the cartilage and the bone of joints. The chronic autoimmune process is initiated by the deposition of immune complexes in the joint and includes different cellular and humoral events. The antigen-specific T-cell response is crucial in the pathogenesis of RA (Gravallese, 2003). T-lymphocytes are activated by two signals. The antigen-specific signal is mediated by the connection of T-cell receptor with the protein-MHC system complex on antigen presenting cells. The second signal is mediated by the costimulation of CD28 receptor presented on native T-lymphocytes by the specific ligand found on the antigen presenting cell. The activated T-cells proliferate and produce cytokines, which can activate other cells participating in the inflammatory reaction (Kremer et al., 2003).

RA is affecting approximately 1% of the whole world population. Patients with RA have a notably reduced life quality (joint and bone degeneration, muscle weakness, persistent pain) and require a long-life therapy. A common effect of a long-term therapy is the development of resistance to treatment and also an increased occurrence of adverse effects. Thus in the therapy of RA, there is a permanent need for new agents concerning also resistance development, occurring in some patients (Bauerova et al., 2011).

Primary and dominant processes in etiopathogenesis of RA are immunological mechanisms, closely related to redox imbalance in the organism, which may potentiate chronic inflammatory processes. Moreover, in inflamed tissue, production of reactive oxygen and nitrogen species (ROS and...
Adjuvant arthritis - an effective tool for preclinical evaluation of antirheumatics.

Adjuvant arthritis (AA) is an animal model of RA in which joint inflammation is triggered by a single intradermal injection of an immunostimulatory agent (adjuvant). The formulation of commercial immunological adjuvants was the result of intense research in the early 20th century. Adjuvant arthritis research can be divided into different phases. In the first phase, in 1947, Jules Freund introduced a mixture of mineral oils, heat-killed mycobacteria and an emulsifying agent, designated complete Freund’s adjuvant (CFA). Later mycobacteria were often omitted, in which case the adjuvant was called incomplete Freund’s adjuvant (IFA). In the second phase, the experimental data inferred that the mycobacterial component and the oil caused arthritis by separate mechanisms. At present, each of the rat and mouse models currently used has clinical features that resemble RA in humans. Histological features of all the models of joint disease include synovial hyperplasia with massive infiltration of inflammatory cells, similar to the histological abnormalities in RA patients. The experimental model of AA belongs to the group of models of erosive arthritis. These rat and mouse models differ among themselves and in comparison to RA with respect to: disease onset, severity of joint inflammation, patterns of joints involved, and various additional clinical and systemic manifestations (Bauerova et al., 2008a). Arthritis induced in rats with intradermal injection of adjuvants containing mycobacteria is an animal model often used for evaluation of potential antirheumatic drugs. This model is also a good methodological tool for investigation of pathological mechanisms in RA. An intradermal injection, into the base of the tail, with heat-killed Mycobacterium tuberculosis in IFA results in destructive arthritis within 14 days in susceptible Dark Agouti or Lewis inbred rat strains. AA can also be induced with cell walls from other bacterial types in IFA, although the arthrogenicity varies (Joe et al., 1999). In many experiments, the induction of arthritis is achieved also with Mycobacterium butyricum in IFA (Akiyama et al., 2005; Bauerova et al., 2006; Bauerova et al., 2009; Nakazato et al., 2005). Basic clinical manifestations of AA are paw swelling, infiltration of mononuclear and polymorphonuclear phagocytes into joints, formation of pannus, periostitis and erosions of cartilage and bone (Williams et al., 1998). The intensity of AA development is described by so-called ‘basic clinical parameters’, i.e. arthritic score or hind paw edema, which are usually measured once a week the complete time of experiment duration. AA has been extensively used for pharmacological testing, and therefore many data exist for comparison in humans. While this model does not mimic perfectly the condition of human arthritis, it is easily reproducible, well defined and has proven useful for the development of new therapies for arthritis, as exemplified also by cytokine blockade therapies (Bendele et al., 1999). AA was used in the evaluation of nonsteroidal inflammatory drugs, such as phenylbutazone and aspirin, during the early 1960s, and later on COX-2 inhibitors, such as celecoxib, were studied. AA in rats shares many features with human arthritis, including genetic linkage, synovial CD4+ cells and T-cell dependence. Nevertheless, one of the major differences between the AA model and human arthritis is simply that the inciting agent is known in the model, though the need for any specific antigen is controversial (Bendele et al., 1999).

Dietary supplements - a source for new arthritis pharmacotherapy.

The development of drugs for RA therapy has been very intensive in recent years. Biological therapy targeted on neutralizing the effect of anti-inflammatory cytokines, particularly TNF-α, IL-1 and IL-6, by using antibodies or soluble receptors provided a great progress in RA therapy. However, not even these expensive drugs are able to cure RA definitely, although they remarkably inhibit the development of arthritis and improve the life quality of patients. Following treatment interruption, a fast development of RA occurs. Biological therapy has also many adverse effects, as development of resistance and secondary infections. For these reasons, the search for new drugs which could avoid these infections or suppress them is still an up-to-date problem. The most frequently applied conventional drug for RA has been methotrexate (MTX). Its application is usually the basic therapy of RA and for patients with poor response to MTX, addition of biological therapy or combination with other conventional drugs is used. Intensive immunosuppressive treatment with MTX or biological therapy critically injures the immunological homeostasis of the organism and increases the risk of infections. This has called for the search for alternative immunomodulatory approaches, which could minimize side effects of immunosuppressive therapy on cellular and humoral immunity. One possibility is represented by the combination of immunosuppressive and immunostimulatory substances or compounds regulating redox balance of the organism. Their application can establish immunological and redox
homeostasis and increase resistance of the organism to infections (Bauerova et al., 2011). Our previous results with different antioxidants and immunomodulators evaluated in adjuvant arthritis (AA) showed beneficial effects of these substances on the development of (AA). Coenzyme Q$_{10}$ in monotherapy improved the clinical parameters of the disease and significantly reduced the oxidative stress during AA. Moreover, addition of coenzyme Q$_{10}$ to standard therapy of arthritis by MTX significantly enhanced its efficacy manifested in improvement of clinical, oxidative stress and immunological markers of the disease (Bauerova et al., 2005; Bauerova et al., 2008b; Bauerova et al., 2010, Bauerova et al., 2011). Curcumin, a well known plant substance, was also studied in AA. AA is accompanied by an increased number of neutrophils in blood and by a more pronounced spontaneous as well as Phorbol 12-Myristate 13-Acetate (PMA) stimulated chemiluminescence. Curcumin was found to be a potent inhibitor of neutrophil functions in experimental arthritis (Jancinova et al., 2009). Further we showed inhibitory properties of carnosine (CARN) against degradation of hyaluronan solutions at experimental conditions in vitro. In the reaction system with the prevalence of •OH and/or peroxy-type radicals, CARN in 200 μmol/L concentration tested exerted a protective action on hyaluronan degradation (Drafi et al., 2010). This in vitro experiment was completed with a preliminary in vivo study in which CARN improved the clinical parameter – hind paw volume, as well as protein carbonyls and T BARS level measured in plasma of rats with AA. We also studied immunomodulatory polysaccharides i.e. glucosamin isolated from \textit{Candida utilis} and β-glucan (Imunoglukán® - IMG) in AA. Both polysaccharides beneficially corrected the clinical markers of AA progression and markers of oxidative stress. Moreover, IMG administration had a positive immunomodulating effect on all cytokine and chemokine plasma levels measured (IL-1, TNF-α, MCP-1 and IL-4) changed markedly due to arthritis progression (Bauerova et al., 2008c; Bauerova et al., 2009, Drabikova et al., 2009). IMG when studied in combination with MTX, markedly potentiated the beneficial effects of MTX on AA progression, which resulted in a more significant reduction of hind paw swelling and arthritic score (Rovensky et al., 2011). Radical-scavenging activity of the water-soluble derivative obtained from the cell wall of baker’s yeast \textit{Saccharomyces cerevisiae} was investigated using the technique of electron paramagnetic resonance. The experiments involved an in vivo study of the activity of carboxymethyl (1,3)-β-D-glucan (CMG) in AA. In this study, a substantial decline of the level of plasmatic carbonyls, a parameter indicating oxidative tissue damage during the progress of arthritic diseases, was observed. We assumed that radical-scavenging properties of CMG could be responsible for its antioxidant activity in the AA model, suggesting possible application of the yeast glucan derivatives in the treatment of arthritis (Kogan et al., 2005). This conception was further applied in experiments with glucomannan in AA, as described below.

**Polysaccharides with immunomodulating properties isolated from mushrooms and plants.**

In recent decades, polysaccharides isolated from botanical sources (mushrooms, algae, lichens, and higher plants) have attracted a great deal of attention in the biomedical arena because of their broad spectrum of therapeutic properties and relatively low toxicity (Tzianabos, 2000). Plant and mushroom polysaccharides reveal immunomodulatory effect that depend on polysaccharide structure and molecular weight (low molecular weight – inhibition, high molecular weight – activation) (Schepetkin and Quinn, 2006). Polysaccharide-rich fungi and plants have been employed for centuries by cultures around the world for their dietary and medicinal benefits (Reynolds, 2003; Hobbs, 2003; Kusaykin et al., 2008; Paulsen, 2001; Yamada and Kiyohara, 1999). Often thought to merely support normal bowel function, blood glucose and lipid levels (Anderson et al., 1994; Weickert and Pfeiffer, 2008; Estruch et al., 2009), certain polysaccharides have attracted growing scientific interest for their ability to exert marked effects on immune system functions, inflammation and cancers (Chan et al., 2009; Lull et al., 2005; Pelley and Strickland, 2000). Ramberg et al. (2010) found 62 publications reporting statistically significant effects of orally ingested glucans, pectins, heteroglycans, glucosamann, fucoidans, galactomannans, arabinogalactans and mixed polysaccharide products in rodents. Fifteen controlled human studies reported that oral glucans, arabinogalactans, heteroglycans, and fucoidans exerted significant effects. Although some papers focused on anti-inflammatory effects, most studies investigated the ability of oral polysaccharides to stimulate the immune system. These studies, as well as safety and toxicity studies, suggest that these polysaccharide products are to be largely well-tolerated.

One of the most important applications of polysaccharides in human medicine has been used in cancer therapy. For millennia, mushrooms have been valued by humankind as an edible and medical resource. A number of bioactive molecules, including antitumor substances, have been identified in many mushroom species. Polysaccharides are the best known and most potent mushroom derived substances with immunomodulating and antitumor properties (Borchers et al., 1999; Mizuno, 1996; Mizuno, 1999; Mizuno, 2002; Lorenzen and Anke, 1998; Ooi and Liu, 1999; Reshetnikov et al., 2001; Tzianabos, 2000; Wasser and Weis, 1999). Historically, hot-water-soluble fractions (decoctions and essences) from medicinal mushrooms, i.e., mostly polysaccharides, were used as medicine in the Far East, where knowledge and practice of mushroom use primarily originated (Hobbs, 2000; Hobbs, 2003; Wasser, 2002). The biochemical mechanisms that mediate the biological antitumor activity of polysaccharides are still not
clearly understood. Polysaccharides from mushrooms do not attack cancer cells directly, producing rather their antitumor effects by activating different immune responses in the host. The antitumor action of polysaccharides requires an intact T-cell component; their activity is mediated through a thymus-dependent immune mechanism (Borchers et al., 1999). Mushroom polysaccharides are known to stimulate natural killer cells, T-cells, B-cells, and macrophage-dependent immune system responses. The immunomodulating action of mushroom polysaccharides is especially valuable as a means of prophylaxis, a mild and non-invasive form of treatment, prevention of metastatic tumors, and as co-treatment with chemotherapy (Wasser, 2002).

D-glucans are one of the largest groups of polysaccharides. Cell-wall polysaccharide D-glucans are branched polymers of D-glucopyranose, either with α- and/or β-linkage configuration. Both α- and β-glucans have been reported in micro-organisms, plants, and animals. The β-glucans are the predominant carbohydrates, making up to more than 50% of the dry weight of the cell wall. The β-glucans (β-1,3-, β-1,4-, and β-1,6-glucose polymers) from diverse sources are different in their structure, chemical, physical, and biological properties, and as a consequence, in their immunomodulatory effects. Moreover, they represent the conserved structure - pathogen-associated molecular pattern (PAMP) - and are effective biological response modifiers, non-specifically enhancing the host immune system by multiple interactions within innate and adaptive mechanisms. The gamut of biological activities of glucans includes direct leukocyte activation, stimulation of phagocytosis, oxidative burst (Sakurai et al., 1992; Wakshull et al., 1999), and stimulation of cell cytotoxicity (cytotoxic T-lymphocytes) (Cross et al., 2001). The stimulated induction of ROS and inflammatory mediators, chemokines, cytokines, and nuclear transcription factors (Adams et al., 1997; Battle et al., 1998; Czop 1986; Majtan et al., 2005; Sakurai et al., 1996) is selectively guided via β-glucan specific receptors on competent cells of the immune system, e.g. complement receptor 3, lactosylceramide, scavenger receptors, and dectin-1 (Brown et al., 2001; Rice et al., 2002). Protective antioxidant and anti-inflammatory activities of carboxylated (1,3)-β-D-glucan isolated from Saccharomyces cerevisiae were reported in AA in Lewis rats (Kogan et al., 2005).

Glucomannans are different sources - chemical structure and application.

Glucomannans are carbohydrate polymers widely distributed in both hardwood and softwood plants, where they have either storage or structural functions. The polymeric sequence is linear and it is composed of (1,4)-beta-D-glucose and (1,4)-beta-D-mannose sugar residues. However, the presence of few short side-chains, which may contain galactose residues, was also reported (Stephen, 1983). Native polymers exhibit some degree of acetylation, which depends on the plant source. The mannose to glucose molar ratio also depends on the plant species, and the content of mannose is usually higher than that of glucose, but 1:1 molar ratios were described (Cescutti et al., 2002). Glucomannans are present in large amounts in the hemicellulose fraction of softwood. They contain chains of randomly arranged β-(1,4)-linked D-mannose and β-(1,4)-linked D-glucose residues in a 3:1 ratio with a degree of polymerization greater than 200 (Northcote, 1972; Popa and Spiridon, 1998). Hardwoods consist of glucomannan with a glucose/mannose ratio of 1:1.5–2 (Hongshu et al., 2002; Timell 1967). The mannose residues of glucomannan provide the branching points in the polysaccharide by 1,6- and/or 1,3-linkages (Aspinall et al., 1962). Glucomannans have a variety of applications, including prevent chronic diseases and weighting control (Ishurd et al., 2006).

The glucomannan which is containing in Amorphophallus species has to date the broadest application in medicine. Amorphophallus konjac (konjac) has long been used in China, Japan and South East Asia as a food source and as a traditional medicine. In traditional Chinese medicine, a gel prepared from the flour has been used for detoxification, tumor-suppression, blood stasis alleviation and phlegm liquefaction; and for more than 2000 years it has been consumed by the indigenous people of China for the treatment of asthma, cough, hernia, breast pain, burns as well as hematological and skin disorders. Over the past two decades, purified konjac flour, commonly known as konjac glucomannan (KGM), has been introduced on a relatively small scale into the United States and Europe, both as a food additive and a dietary supplement. The latter is available in capsule form or as a drink mix and component of food products. Clinical studies demonstrated that supplementing the diet with KGM significantly lowered plasma cholesterol, improved carbohydrate metabolism, bowel movement and colonic ecology. Standards for the classification of both konjac flour and KGM have been established by the Chinese Ministry of Agriculture, the European Commission and the U.S. Food Chemicals Codex. However, to date, there is no worldwide agreed regulatory standard for konjac flour or KGM (Chua et al., 2010). The mannans isolated from Amorphophallus species contain glucomannan associated with starch-like α-glucan (Aspinall, 1959). They were described to comprise 70% of 1,4-linked β-D-mannopyranose and 30% of β-D-glucopyranose residues and may have a role as food reserve. Dimers and trimers obtained by enzymatic hydrolysis of the glucomannan produced by the plant Amorphophallus konjac were analyzed in order to obtain information on the saccharidic sequences present in the polymer. The polysaccharide was digested with cellulase and β-mannanase and the oligomers produced were isolated by means of size-exclusion
Glucosylns from Candida utilis.

A new chemotype glucosann isolated from Candida utilis (GM) mutant was further studied with the aid of methylation analysis and fragmentation analysis by controlled acetylation. It was thus revealed that the GM had an α-(1,6) linked D-mannosyl backbone partially substituted with side chains of one, two, three, or four D-mannosyl units connected by α-(1,2) linkages; moreover, it has an additional side chain in which D-glucose residues are linked through an α-(1,6) linkage at the non-reducing ends of four D-mannosyl units (Ogawa et al., 1988). This GM was further studied by inhibition of the homologous precipitin reaction with oligosaccharidies obtained from the GM by partial acid hydrolysis and controlled acetylation. Oligosaccharidies having at least two consecutive α-(1,2)-linked mannose residues at the non-reducing end and gluco-mannopentasaccharide were effective inhibitors. Thus it appears that the GM had two groups of antigenic determinants, one corresponding to the side chains of two, three, and four mannose units connected by α-(1,2)-linkage, and the other corresponding to a side chain composed of an O-α-D-glucopyranosyl-(1,6)-O-α-D-mannopyranosyl-(1,2)-O-α-D-mannopyranosyl-(1,2)-D-mannose unit (Ogawa et al., 1990). Kogan et al. (1993) investigated the structure of the GM isolated from the cell walls of Candida utilis using acetylation fragmentation, methylation analysis and NMR spectroscopy. The structure of the GM resembles that of the cellular mannans of other Candida species, except that the longer tetra- and penta-saccharide side-chains are terminated with a glucosyl residue. The presence of the non-reducing glucosyl groups at the ends of the side-chains caused the Candida utilis not to cross-react in a double immunodiffusion test with other Candida species that possess mannan antigens and cross-react with Hansenula species with GM antigens.

Finally, besides the chemically synthesized derivatives of glucosann, there is a class of natural derivatives of glucosann, such as those obtained from the yeast Candida utilis. This variety of GM is characterized by the presence of phosphate groups, a high content in mannose (mannose:glucose ratio 11.4:1) and α-1,6 linkages in the main backbone. Moreover, in contrast to the ordinary GM, the phosphorylated derivative presents low molecular weight (around 150 kDa) and high solubility in water and polar solvents, such as acetone and ethanol. The negative charge of this derivative is a very attractive characteristic for its application in the pharmaceutical field. For example, our group has extensively investigated the formation and drug delivery applications of nanoparticles, made of chitosan and phosphorylated glucosann (Alonso-Sande et al., 2006; Cuna et al., 2006).

GM isolated from Candida utilis with molecular weight 30 kDa was administered either intraperitoneally or orally.
prior to cyclophosphamide (CP) injection and its effect on the frequency of micronuclei was evaluated in polychromatic erythrocytes of mouse bone marrow. GM administration by either route decreased significantly (p<0.002) the clastogenic effect of CP. The protective effect was concentration-dependent, with a higher decrease achieved by 200 mg/kg than by 100 mg/kg body weight. The fact that GM was effective also at oral administration is indicative of the passage of GM molecules through the wall of the gastrointestinal tract. The important characteristics of GM, such as good water solubility, relatively small molecular weight (30 kDa), and antimutagenic effect exerted also at oral administration, appear to be promising features for its use as a natural protective agent (Chorvatovicova et al., 1999). Further potent antimutagenic, anticlastogenic, and bioprotective activities of GM against chemical compounds with different modes of action were documented (Vlckova et al., 2004). A different study described GM due to its efficient action as an antimutagen, anti-clastogen, DNA-break inhibitor or inducer, and as a cytotoxic/cytostatic effect enhancer. Several possible mechanisms of the observed bioprotectivity, including free radical scavenging, have been suggested (Miadokova et al., 2006). In one of the recent studies, a highly branched GM, a member of the α-(1,6)-D-mannan group, was tested for its photoprotective effects in an in vitro model of UVB-irradiated human keratinocytes and an in vivo model of UV-induced erythema formation in human volunteers. GM suppressed the UVB-induced decrease of keratinocyte viability, which was connected with the suppression of UVB-induced keratinocyte apoptosis. GM reduced UVB-mediated caspase activation together with suppression of DNA fragment release into the cytoplasm. Furthermore, GM suppressed UVB-induced gene expression of pro-inflammatory markers including nuclear factor kappa B, inducible nitric oxide synthase, interleukins 8 and 1, together with suppression of prostaglandin E2 and interleukin 1α protein release. In vivo, GM decreased UV-induced skin erythema formation, which was correlated with a decrease of phospholipase A2 activity within the stratum corneum. It was concluded that GM isolated from Candida utilis possessed significant photoprotective effects on human keratinocytes in vitro as well as in vivo (Ruszova et al., 2008).

Glucosmanan from Candida utilis evaluated in adjuvant arthritis.

Immunomodulators with antioxidant activity have been investigated in adjuvant arthritis (AA) on Lewis rats. Protective antioxidant and anti-inflammatory activities of carboxylated (1,3)-β-D-glucan isolated from Saccharomyces cerevisiae were reported (Kogan et al., 2005). GM from Candida utilis was evaluated in the same model. The anti-arthritic activity for cell-wall GM was associated with antioxidant activity in vivo (Bauero et al., 2006; Mihalova et al., 2007; Bauero et al., 2008c; Bauero et al., 2009; Drabikova et al., 2009).

In this experiment we were focused on assessing the contribution of the in vivo antioxidant properties of GM on its anti-arthritic effect. For this purpose not only biochemical methods were used but also chemiluminiscence measurements were performed in whole blood and in selected tissues, as spleen and hind paw. Additionally, the functionality of neutrophils was evaluated by flow cytometry.

MATERIALS & METHODS

Glucosmanan from Candida utilis preparation and characterization.

As a biological source of yeast glucosmanan Candida utilis was used. Strain CCY 29-38-18 was obtained from the Collection of Yeast and Yeast-like Microorganisms (Institute of Chemistry, Slovak Academy of Sciences, Bratislava). The yeast (Candida utilis) was cultivated in 2% glucose semi-synthetic liquid medium for 70 hrs at 28°C. The cell biomass was separated and washed by centrifugation. Cells were suspended in 0.2 mol/L NaCl and the mixture was extracted by autoclaving repeatedly with saline. After separation of the biomass by centrifugation, the supernatants were concentrated and the cell wall glycoproteins were precipitated with three-fold volume of ethanol. GM was isolated from glycoproteins using extraction with 2% KOH and purification with Fehling reagent as described previously (Kogan et al., 1988). Elemental analysis revealed 0.075% nitrogen. Molar mass of polysaccharide was 33 kDa, as determined by gel filtration. According to specific optical rotation measurement, a prevalent α-D-glycosidic linkage was observed. IR spectrum contained absorption bands at 810 and 970 cm⁻¹ that are characteristic of α-D-mannans (Kato et al., 1973). The 13C spectra and the carbon pikes of this polysaccharide correspond with the GM substance determined by Kogan et al. (1993).

Animals, design of the experiment and treatment with glucosmanan.

Male Lewis rats, weighing 160–180 g, were obtained from the Breeding Farm Dobrá Voda (Slovakia). The rats had free access to standard pelleted diet and tap water. The animal facilities comply with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Purposes. The experimental protocol was approved by the Ethics Committee of the Institute of Experimental Pharmacology and Toxicology and by the Slovak State Veterinary Committee of Animal Experimentation. AA was induced by a single intradermal injection of heat-inactivated Mycobacterium butyricum in incomplete Freund’s adjuvant (Difco Laboratories,
Detroit, MI, USA). The injection was performed near the tail base. The experiments included healthy animals as controls (CO), arthritic animals without any drug administration (AA), and arthritic animals with GM administration (once daily in the oral dose of 7.5 (AA-GM1) and 15 mg/kg (AA-GM2), over a period of 28 days).

**Blood and tissues collection.**

Blood was collected under light ketamin/xylasine anesthesia (ketamin - Narkamon® 5% 50 ml sol. inj. SPOFA, Czech Republic and xylasine - Rometar® 2% 50 ml sol. inj. SPOFA, Czech Republic) from the retro-orbital plexus into lithium heparin (Sarstedt Multivette) blood sample collectors on days 7 and 28 and flow cytometric measurements were immediately performed. After the animals had been sacrificed under deep ketamin/xylasine anesthesia, blood for chemiluminiscence measurement and plasma preparation, tissues for spleen and hind paw joint homogenate preparation as well as for isolation of mitochondria from skeletal muscles were taken on day 28. Heparinized plasma was stored at –70 °C until biochemical analysis was performed.

**Evaluation of clinical parameters: hind paw volume and body weight of animals.**

Hind paw volume (HPV) increase was calculated as the percentage increase in HPV on a given experimental day relative to the HPV at the beginning of the experiment. HPV was recorded on days 1 and 28 with the use of an electronic water plethysmometer (UGO BASILE, Comerio-Varese, Italy). Change of body mass - CBM (g) was calculated as the difference of body weight measured on day 28 and the body weight measured at the beginning of the experiment.

**Analysis of total antioxidant status (TAS).**

Incubation of ABTS® (2,2’-azinobis(3-ethylbenzothiazoline-6-sulfonate)) with peroxidase (metmyoglobin) results in the production of the radical cation ABTS+. This species is bluegreen in color and can be detected at 600 nm. Antioxidants in the sample cause inhibition of this color production to a degree that can be detected. Aliquots of whole blood (lithium heparin) (30 µl) were measured according to the manufacturer’s instructions (RANDOX® TAS kit) using a microplate reader (Labsystems Multiskan RC). According to Miller et al. (1993) the TAS measurement procedure with RANDOX® TAS kit has been applied to physiological antioxidant compounds and radical-scavenging drugs, and an antioxidant ranking was established, based on their reactivity relative to a 1.0 mmol/L Trolox standard. The producer declares that RANDOX® TAS kit possesses a wide measuring range (0-2.5 mmol/L), intra-assay C.V. of 1.2% and inter-assay C.V. of 2.4%.

**Measurement of thiobarbituric acid reactive substances (TBARS) in plasma.**

The reaction with TBA occurs by attack of the monoenoic form of malondialdehyde (MDA) on the active methylene groups of TBA. Visible and ultraviolet spectrophotometry of the pigment confirms the primary maximum at 535 nm. TBARS were measured in heparinized blood plasma. The amount of 750 µL of 0.67 % TBA (Merck), 750 µL of 20 % trichloroacetic acid (Fluka), and 350 µL of phosphate buffer (pH 7.4) were added to 150 µL of plasma, then mixed and incubated in a water bath at 90 °C for 30 min. The reaction was stopped by dipping the test tubes into ice for 10 min. Samples were centrifuged at 3000 rpm (centrifuge Eppendorf 5702 R, Germany). The supernatant was removed and absorbance measured at 535 nm (Specord 40, Jena, Germany) in a 0.5 cm cuvette (Brown and Kelly, 1996).

**Determination of whole blood chemiluminescence and neutrophil count.**

The total production of oxidants in neutrophils (spontaneous and stimulated with PMA) was determined on the basis of luminol-enhanced chemiluminescence, using a microplate luminometer Immunotech LM-01T (Czech Republic). Samples contained 50 µl aliquots of luminol (250 µM), 200x diluted rat blood, horse radish peroxidase (8U/ml) and PMA (0.01 µM) or phosphate buffer. Data were based on integral values of chemiluminescence over 3600 s. (RLU x s; RLU, relative light units). Neutrophil count was assessed by Coulter Counter (Coulter Electronics, England). Before counting, whole blood was diluted 2000x and erythrocytes were destroyed by lysing reagent (Drabikova et al., 2009, Jancinova et al., 2009).

**Immuno-cytometric evaluation of simultaneous phagocytosis and oxidative burst.**

Flow cytometric analysis was used for these measurements, according to the method published by Kroner et al. (2010) and modified for the model of AA (Bauerova et al., 2010). Phagocytosis accompanied by a respiratory burst of rat granulocytes was evaluated by flow cytometry (Beckman-Coulter FC 500 flow cytometer, CXP software). For each sample, a fluorescence histogram of 10 000 cells was generated and analyzed. Gates were set around the granulocytes population to exclude debris. Measurements of phagocytosis, i.e. the ingestion of bacteria took place under controlled conditions using a fluorescein-labeled opsonized Staphylococcus aureus (SPA-FITC) (Molecular Probes, The Netherlands). The metabolic activity was determined via the oxidative burst of the stimulated transformation of originally non-fluorescent hydroxyethidine (HE) (Polysciences, USA) to fluorescent ethidium, which intercalates DNA to afford red fluorescence (excitation of 488 nm) following SPA-FITC ingestion. Aliquots of whole blood (lithium heparin) (30 µl) from rats were incubated with HE (15.75 mg in 5 ml of
dimethylformamide, Merck, Germany) for 15 min at 37 °C. Following treatment with SPA-FITC for 15 min at 37 °C, the reaction was stopped with ice. A subsequent lysis was performed for 15 min with an ice-cold ammonium-chloride-potassium chloride (ACK) lysis buffer (200 mL deionized water; 1.658 g NH₄Cl; 0.2 g KHCO₃; 7.4 mg Na₂EDTA; pH 7.2–7.4). The mean % of phagocytic cells represents the percentage of granulocytes ingesting at least one SPA-FITC particle; mean % of respiratory burst represents the percentage of granulocytes tagged by ethidium and the mean metabolic activity % represents the percentage of granulocytes which ingested at least one SPA-FITC and were tagged by ethidium.

Analysis of tissue activity of cellular γ-glutamyltransferase.

The activity of cellular γ-glutamyltransferase (GGT) in hind paw joint and spleen tissue homogenates was measured by the method of Ondrejickova et al. (1993). The samples were homogenized in a buffer (2.6 mM NaH₂PO₄, 50 mM Na₂HPO₄, 15 mM EDTA, 68 mM NaCl; pH 8.1) at 1:9 (w/v) by UltraTurrax TP 18/10 (Janke & Kunkel, Germany) for 1 min at 0 °C. Substrates (8.7 mM γ-glutamyl-p-nitroanilide, 44 mM methionine) were added to 65% isopropylalcohol in final concentrations of 2.5 mM and 12.6 mM, respectively. After incubation for 60 min at 37°C, the reaction was stopped with 2.3 ml cold methanol and the tubes were centrifuged for 20 min at 5000 rpm. The absorbance of the supernatant was measured in a Specord 40 (Jena, Germany) spectrophotometer in a 0.5 cm cuvette at 406 nm. Reaction mixtures in the absence of either substrate or acceptor were used as reference samples.

Reactive oxygen species (ROS) generation in selected tissues determined by luminol-enhanced chemiluminiscence.

On day 28, ROS generation in the spleen and hind paw joint (cartilage and soft tissue without bone) was determined by luminol-enhanced chemiluminiscence (CL) (Drabikova et al., 2007; Drabikova et al., 2009). Briefly, the pieces of approximately 20 mg spleen and 450 mg joint wet weight were dissected. The samples were placed into preoxygenated (95% O₂ and 5% CO₂) physiological saline solution - PSS (in mM: 122 NaCl, 5.9 KCl, 1.2 MgCl₂, 1.25 CaCl₂, 15.0 NaHCO₃, and 11 glucose, pH 7.4), and transferred into a cuvette containing 1.0 ml PSS with luminol (final conc. 400 µM) immediately prior to assessment of ROS generation. CL responses were measured at 37°C and recorded continuously for 10 min in a lumi-aggregometer model 500 (Chrono-log Corp., USA) at appropriate sensitivity setting. The wet weight of samples was recorded at the end of experiment. Data were evaluated as the peak of the CL curve expressed as mV/100 mg wet weight of joint and mV/1 mg wet weight of spleen.

Determination of CoQ₉ and CoQ₁₀ levels in mitochondria of the skeletal muscles.

Concentrations of oxidized forms of coenzyme Q₉ and Q₁₀ were determined by isocratic high-performance liquid chromatography (HPLC, LKB, Sweden) according to Lang et al. (1986) with some modifications (Kucharska et al., 1998). Mitochondrial suspension was vortexed twice for 5 minutes with 2 ml of the mixture of hexane/ethanol (5/2, v/v, Merck, Germany). Collected organic layers were evaporated under nitrogen, the residues were taken up in ethanol and injected into Separon SGX C18 7 µm 3x150 mm column (Tessek, Czech Republic). Elution was performed with methanol/acetonitrile/ethanol (6/2/2, v/v, Merck, Germany). The concentrations of coenzyme Q₉ and Q₁₀ were detected spectrophotometrically at 275 nm, using external standards (Sigma, Germany). Data were collected and processed using CSW 32 chromatographic station (DataApex Ltd, Czech Republic). Concentrations of compounds in plasma were calculated in nmol.mg prot⁻¹.

Isolation of mitochondria.

Mitochondria from hind paw skeletal muscle tissue were isolated by means of differential centrifugation in isolation solution containing 180 mmol.¹ KCl, 4 mmol.¹ EDTA and 0.1% of bovine serum albumin, according to Sarma et al. (1976) with slight modifications. Tissue was minced and homogenized in the isolation medium using a teflon-to-glass homogenizer at 4°C. Protease type VIII (Sigma-Aldrich) in the dose 2.5 mg/g of the tissue was added into the isolation solution during mixing for 20 min. The homogenate was centrifuged at 700 g for 10 min, the supernatant was decanted and centrifuged twice at 5600 g for 10 min. The mitochondrial pellet was washed twice in isolation solution without albumin. Mitochondrial proteins were estimated by the method of Lowry et al. (1951).

Statistical analysis.

The data were expressed as arithmetic mean ± SEM, with 8 – 10 animals in each experimental group. The untreated arthritis group was compared with healthy control animals (*), treated arthritis groups were compared with untreated arthritic animals (+). For significance calculations unpaired Student’s t-test (two sample, unequal variance) was used with the following significance designations: extremely significant (p < 0.001), highly significant (p < 0.01), significant (p < 0.05), not quite significant (p = 0.05), not significant (p > 0.05).

RESULTS & DISCUSSION

RA is a common severe joint disease that involves all age groups. The pathogenesis of RA is associated predominantly with the formation of free radicals at the site of inflammation. The inflammatory process develops in the tissue of the synovium: primary sources of reactive oxygen...
species (ROS) in RA are leukocytes, which are recruited to accumulate within the synovium. Oxidants can be produced by activated macrophages in the synovial membrane and by activated neutrophils in the synovial cavity (Firestein et al. 1997, Firestein 2003). The control of inflammation in RA patients by natural and synthetic substances with anti-inflammatory and/or antioxidant and immunomodulatory effects, which are safe also during long-term administration, could become a relevant part of RA therapy. Modulation of oxidative stress (OS) accompanying RA can offer a new approach and crucially modify treatment of this disease (Bauerova and Bezek, 1999; Bauerova et al., 2011).

Prokopova et al. (1993) were the first to describe a therapeutic effect of simple carbohydrates on AA. We were the first to report on the protective antioxidant and antiinflammatory activities of carboxylated (1,3)-beta-D-glucan isolated from *Saccharomyces cerevisiae* in Lewis rats with AA (Kogan et al., 2005). Glucomannan (GM) from *Candida utilis* was evaluated in the same model. The anti-arthritic activity of cell-wall GM was associated with antioxidant activity in vivo (Bauerova et al., 2006; Mihalova et al., 2007). In the following experiment, the beneficial action of GM was revealed mainly in HPV decrease. Further, a decrease of the activity GGT in the spleen, hind paw joint and muscle tissue homogenates, decrease of the plasmatic activity of N-acetyl-β-D-glucosaminidase, and finally suppression of lysozyme and peroxidase activity assessed in peritoneal macrophages were observed in arthritic animals treated with GM. All these findings speak in favor of the antiinflammatory activity of GM. Moreover, a significant improvement of the arthritis-induced suppression of total antioxidant status was detected along with a decrease of the level of the arthritis-associated protein carbonyls in plasma. In this experiment, two GM doses were successfully evaluated – 5 and 7.5 mg/kg b.w.. Peroral and intraperitoneal ways of administration were also compared (Bauerova et al., 2008c). In another study, we tested the effect of GM in a higher dose of 15 mg/kg b.w. administered orally. On day 28 after *Mycobacterium butyricum* induced AA, GM was found to reduce HPV. Neutrophil count in whole blood was significantly increased on day 28 after induction of AA, yet GM in the dose of 15 mg/kg b.w. did not change it significantly. The spontaneous and PMA-induced chemiluminiscence (CL) was significantly increased in whole blood of rats with AA in comparison with healthy controls. GM, 15 mg/kg b.w., decreased spontaneous as well as PMA-stimulated CL. CL of spleen and joint in rats with AA was significantly increased in comparison with controls. GM significantly decreased CL of joints, while CL of the spleen was not affected. The obtained results showed that GM reduced ROS generation in arthritic rats. The predominant decrease of extracellular ROS production suggests a protective effect of GM against tissue damage, especially in the hind paw joint of arthritic rats (Drabikova et al., 2009). Further we decided to compare the effect of GM and Imunoglukan®, a β-(1,3/1,6)-D-glucan (IMG), which was isolated from *Pleurotus ostreatus*. Both GM as IMG were effective in reducing HPV and improving the oxidative status (Bauerova et al., 2009).

**FIGURE 1.** Hind paw volume of animals measured on day 28. The data were expressed as arithmetic mean with SEM. Each group contained 8-10 animals. Statistical significance was evaluated using unpaired Student’s t-test: ***P<0.001 with respect to control healthy animals; +P<0.05 and nqP=0.05 with respect to untreated arthritic animals.

**FIGURE 2.** Changes in body weight of animals on day 28. The data were expressed as arithmetic mean with SEM. Each group contained 8-10 animals. Statistical significance was evaluated using unpaired Student’s t-test: **P<0.01, and ***P<0.001 with respect to untreated arthritic animals.

In the present experiment, we were focused on revealing the contribution of antioxidative properties of GM to its observed anti-arthritic effect monitored via clinical parameters as change of hind paw volume and change of animals’ body weight. Two doses of GM were tested – 7.5 and 15 mg/kg b.w.. Both doses were administered in the same experimental design: orally, daily until end of the experiment on day 28. Figure 1 and 2 show a comparison of the effect of both GM doses on clinical parameters. Improvement of increased HPV and decreased animal body weight were clearly demonstrated. However, the effect of GM on CBW was more pronounced than its effect on HPV change. Both doses of GM modulated therapeutically the clinical parameters changed due to AA – HPV and CBM.
This observation proved the antiarthritic effect of GM in AA, already referred to in our previous papers (Bauerova et al., 2006; Bauerova et al., 2008c; Bauerova et al., 2009; Mihalova et al., 2007).

The GM antioxidant action in vivo was studied as its systemic effect (whole blood chemiluminescence, TAS and TBARS levels in plasma) and locally (chemiluminescence and GGT activity measured in spleen and hind paw tissue, levels of CoQ9 and CoQ10 in mitochondria of skeletal muscles). Additionally, also the functionality of neutrophils by flow cytometry was evaluated.

According to the published results (Bauerova et al., 2008c), GM does not affect the peroxidation of lipids as TBARS levels remain unchanged after GM treatment (Fig.3-left). OS, a consequence of chronic inflammatory processes occurring in AA, increased after experimental day 14, which marked also the onset of clinical manifestations of the disease. OS increased the consumption of endogenous antioxidants in plasma and thus caused a lowering of the plasma antioxidant capacity, measured as the total antioxidant status with RANDOX TAS kit (Bauerova et al., 2009, Mihalova et al., 2007). In the present experiment, we measured the TAS on experimental day 28. GM in the dose range tested led to a decrease of the systemic OS caused by AA, however on using the lower dose of 7.5 mg/kg b.w., the TAS values were significantly returned to the control values (Fig.3-right).

There are multiple experimental studies dedicated to neutrophil-generated chemiluminescence of whole blood (Arnhold et al., 1994; Cedergren et al., 2007; Miesel et al., 1996) and of synovial fluid (Arnhold et al., 1994; Cedergren et al., 2007), depending on the disease severity in patients with RA. An increase in whole blood chemiluminescence (2–8 fold) was shown in RA patients compared with healthy volunteers (Miesel et al., 1996). Animals with AA had significantly elevated spontaneous chemiluminescence from the seventh experimental day until the end of experiment (day 28). Neutrophils in whole blood of AA animals reacted excessively to stimulation with PMA and produced 6–9 times more ROS. The development of AA in rats was also accompanied with an increase in blood neutrophil count, when compared with control animals (Nosal et al., 2007). In the present experiment we analyzed day 28 to measure the effect of GM on spontaneous chemiluminescence and on chemiluminescence stimulated with PMA. For both types of chemiluminiscence, OS directly associated with neutrophil burst was markedly diminished on applying a larger dose of 15 mg/kg b.w.. For the lower GM dose, no significance was achieved, however a slight chemiluminiscence decrease was shown (Fig.4). As TAS was more influenced by the lower GM dose, the TAS and chemiluminiscence results evaluated together may imply that more mechanisms could be involved in the
development of OS in AA, which may be also neutrophil-independent. Neutrophil count in whole blood was significantly increased on day 28 after induction of AA, yet GM did not change it significantly (Fig. 5).

FIGURE 5. Number of neutrophils in whole blood of animals on day 28. The data were expressed as arithmetic mean with SEM. Each group contained 8-10 animals. Statistical significance was evaluated using unpaired Student’s t-test: ***P<0.001 with respect to control healthy animals.

In the present study, we therefore decided to investigate this finding more precisely using flow cytometry. Another reason was that the changes in neutrophils occurred before the clinical parameter HPV started to be increased. Due to arthritis, both phagocytosis and oxidative burst were already significantly increased on experimental day 7 (Fig. 6). Metabolic activity of neutrophils, as the percentage of double positive cells (simultaneously phagocytic and positive for oxidative burst), was decreased (Fig. 6). This finding could be explained by an increased number of “arthritic” neutrophils, which are positive only for oxidative burst and therefore are not counted as double positive cells.

All flow-cytometric measurements were performed for the lower GM dose only. Changes in the functionality of granulocytes during the development of AA with respect to oxidative burst, metabolic activity and phagocytosis following the GM p.o. application to AA rats revealed significant immunomodulatory trends (Fig. 6 and 7). The acceleration of phagocyte followed by potentiation of oxidative burst response by soluble GM was described elsewhere (Hajkova et al., 2009). Due to the persisting inflammatory nature of AA towards a chronic stage and the immunostimulating behavior of GM, the most significant anti-inflammatory efficacy of GM immunotherapy of AA was observed on day 28, the improvement of phagocytosis being 20.37% in comparison with the AA untreated control group (Fig. 7). This beneficial trend was accompanied also with a 9.35% improvement of oxidative burst in the GM-treated group. The metabolic activity comprising both phagocytic response and oxidative burst response revealed non-significant improvement and return to normal control status (Fig. 7). Moreover, in comparison with day 7, the beneficial lowering of phagocytosis represented 3.74% and of metabolic activity 7.58% (Fig. 6 and 7). A significant decreasing trend following GM intervention was also observed with oxidative burst, the decrease between day 7 and day 28 represented 40.77%, suggesting an important anti-inflammatory impact of GM treatment (Fig. 6 and 7).

Therefore, it could be assumed, that Candida utilis cell-
wall GM is a potential immunomodulative agent in treatment of the exaggerated neutrophilic inflammation accompanying arthritis.

FIGURE 7. Functionality of neutrophils represented by phagocytosis, oxidative burst and metabolic activity assessed on day 28. Changes in all groups with arthritis were calculated with respect to the control value assessed for healthy control animals. The dot-and-dashed line graphically represents the value of control as 100%. The data were expressed as arithmetic mean with SEM. Samples from 8-10 animals were used in each group. Statistical significance was evaluated using unpaired Student’s t-test: *P<0.05 and ***P<0.001 with respect to control healthy animals; +P<0.05 and nqs - P=0.05 with respect to untreated arthritic animals.

FIGURE 8. Reactive oxygen species generation in spleen and joint tissues of animals determined by a method of luminol-enhanced chemiluminiscence on day 28. The data were expressed as arithmetic mean with SEM. Samples from 8-10 animals were used in each group. Statistical significance was evaluated using unpaired Student’s t-test: nqs - P=0.05 and **P<0.01 with respect to control healthy animals; +P<0.05 and nqs - P=0.05 with respect to untreated arthritic animals.

Oxidative damage to the tissues was demonstrated in AA - ROS levels in joint and spleen were analyzed by chemiluminiscence (CL) assessment (Drabikova et al., 2009). CL of spleen and joint in rats with AA was increased in comparison with controls (3.38±1.07 mV/1mg wet weight vs. 1.33±0.16 mV/1mg wet weight, and 6.63±1.34 mV/100mg wet weight vs. 1.11±0.11 mV/100mg wet weight), (Fig.8). GM decreased CL of joints (Fig.8-right), while CL of the spleen was not affected by GM (Fig.8-left). The higher dose was more effective. The obtained results showed that GM reduced ROS generation in arthritic rats on local level.

GGT is an important component of inflammatory processes since its activity is closely connected with the overall antioxidant status of the organism. In our experiments, GGT in the joint from the hind paw (cartilage and soft tissue without bone) and in spleen tissue was determined at the end of the experiment, on day 28. In our previous experiments we found that the activity of GGT was approximately 3–6 times higher in AA animals than in healthy controls in the spleen and 1.4–2.3 higher in the joint (Bauerova et al., 2006; Bauerova et al., 2008b; Bauerova et al., 2009; Sotnikova et al., 2009). The data shown in Figure 9 are in good agreement with these findings. The fact that the GGT activity was elevated also in peripheral joint tissue is in good agreement with clinical studies of patients with RA, who had increased levels of GGT not only in serum and urine but also in synovial fluid (Rambabu et al., 1990). In one of our studies we showed a good correlation between GGT activity in joint tissue and the HPV of arthritic animals (Bauerova et al., 2006). The results of chemiluminiscence and on GGT activity measured in tissues of spleen and of hind paw were very close. For GGT activity we observed the same trends – spleen tissue was not affected by GM treatment (excluding the lower GM dose) (Fig.9-left) and hind paw tissue was significantly and dose-independently affected (Fig.9-right). GGT activity and chemiluminiscence assessed in hind paw tissue increased at the progression of arthritis and were significantly suppressed after treatment with GM. This finding indicates a local antioxidant activity exerted by GM in hind paw tissue selected from the joint area.

FIGURE 9. Activity of GGT in spleen and joint homogenates of animals measured on day 28. The data were expressed as arithmetic mean with SEM. Samples from 8-10 animals were used in each group. Statistical significance was evaluated using unpaired Student’s t-test: ***P<0.001 with respect to control healthy animals; +P<0.05, ++P<0.01 and nqs - P=0.05 with respect to untreated arthritic animals.

FIGURE 10. Levels of CoQ₉ and CoQ₁₀ in skeletal muscle mitochondria of animals on day 28. The data were expressed as arithmetic mean with SEM. Samples from 8-10 animals were used in each group. Statistical significance was evaluated using unpaired Student’s t-test: *P<0.05 and **P<0.01 with respect to control healthy animals; +P<0.05, ++P<0.01 and +++P<0.001 with respect to untreated arthritic animals.
Previously we analyzed the plasma level of one of the most important endogenous antioxidants in rats – coenzyme Q9 (CoQ9). Significant changes in the levels of CoQ9 and/or CoQ10 have been noted in a wide variety of diseases in both animal and human studies. These changes may be caused by impairment in CoQ biosynthesis or excessive utilization of CoQ by the body, or any combination of these processes (Bauerova et al., 2008b; Littarru et al., 1991). Plasmatic concentration of CoQ9 in rats is about 10 times higher than the concentration of CoQ10 (Dallner & Sindelar, 2000). In AA the arthritis process increases significantly the plasma level of CoQ9 in comparison with healthy controls. Evidently, the arthritic processes stimulate the synthesis of CoQ9 and its transport to plasma (Bauerova et al., 2010). In the present experiment, we were focused on the measurement of CoQ9 and CoQ20 levels in mitochondria of skeletal muscles. Levels of both forms of CoQ were increased due to arthritic disease but returned to the control values upon GM administration. Significance and dose-independence was found (Fig.10). Moreover, also on the local level, similarly to plasma, the concentration of CoQ9 was approximately 10 times higher as that of CoQ10.

In conclusion, the anti-arthritic effect of GM in AA was evidenced. Its in vivo antioxidant activity has a large impact on this effect, both in the whole system and locally. The local effect of GM was primarily exerted in the hind paw tissue and mitochondria of skeletal muscles. As to the intensity of GM effects observed for the tested doses of 7.5 and 15 mg/kg b.w., no significant difference was manifested; however the lower dose was more effective concerning the number of parameters affected. The results suggest that Candida utilis cell-wall GM is a potential immunomodulative agent in treating exaggerated neutrophilic inflammation accompanying arthritis and thus GM seems to be beneficial for RA therapy. Final decisions for its practical use as dietary supplement in RA call for further more detailed research not only in preclinical but also in clinical conditions.

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